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A NEW WATER SAMPLE SHIPPING CASE.

WITH SOME OBSERVATIONS ON THE CHANGES THAT TAKE PLACE IN STORED SAMPLES OF WATER.

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Shortly after the establishment of the laboratory of the sanitary district of the Great Lakes at Chicago in 1915, it was found that for the proper carrying on of the work of this district it would be necessary to ship water samples from points as far away as Buffalo, N. Y., and Duluth, Minn. This necessitated a shipping case which would maintain the samples at a low temperature for a period of at least 48 hours.

Information was obtained from several State boards of health as to the type of shipping case used by them and the results obtained therefrom. It was found that most of the cases in use were constructed of wood and insulated with hair felt. The majority of them would not hold a sufficient amount of ice to keep the samples below 10° C. for 48 hours. Furthermore, as a general rule, they were not designed to hold more than two 4-ounce sample bottles or four 2-ounce sample bottles. The only case which would maintain a low temperature for the requisite time was patented, and the main objection to this case was that it was designed to hold only four 2-ounce sample bottles.

The work to be carried on at the Chicago laboratory consisted largely of bacteriological examinations of samples of water collected from lake vessels, and it was important that a considerable number of these samples be collected from the larger ports during the few months during which navigation was at its maximum intensity. It was therefore evident that a shipping case to answer the purpose should be capable of holding at least eight 4-ounce sample bottles, and should have a sufficient ice capacity to maintain them at a temperature of not more than 10° C. for 48 hours.

With these requirements in mind, a case was designed which has proved entirely satisfactory.

The experience of other laboratories using wooden cases showed that these cases went to pieces rather rapidly under the severe handling received from express companies. It was therefore deemed advisable to design a case wholly of metal which would stand up under the severe traffic conditions to which it would be subjected. A study of insulating materials showed that pressed corkboard would provide the greatest amount of insulation with the least amount of space.

The details of construction as finally worked out are as follows: The case is constructed of galvanized iron with pressed corkboard insulation. The outside of the case is made of 20-gauge galvanized iron and measures 15 inches in length, 8 inches in width, and 13 inches in depth. The pressed corkboard is 1 inch in thickness and is inclosed within an inner lining of 24-gauge galvanized iron, which makes a water-tight joint with the outer casing. The inside dimensions are 1 inch less than the outside dimensions. The insulated tapering cover of the dimensions shown in figure 1 drops into the inside of the case. Over this cover fits a second cover of 22-gauge galvanized iron, hinged on one side and with a padlock hasp on the other. This outside cover is wired entirely around its lower edge to stiffen it. Within the outside case, held in place by a circular band of galvanized iron, is a second can. This can is made of 26-gauge galvanized iron, is 5 inches in diameter and 10 inches in depth. It has a slip cover, the sides of which are 2 inches in depth. The detailed dimensions and form of construction are shown in figures 1 and 2. On each end of the case is riveted a heavy piece of strap iron through which is inserted a rope handle. This handle makes the

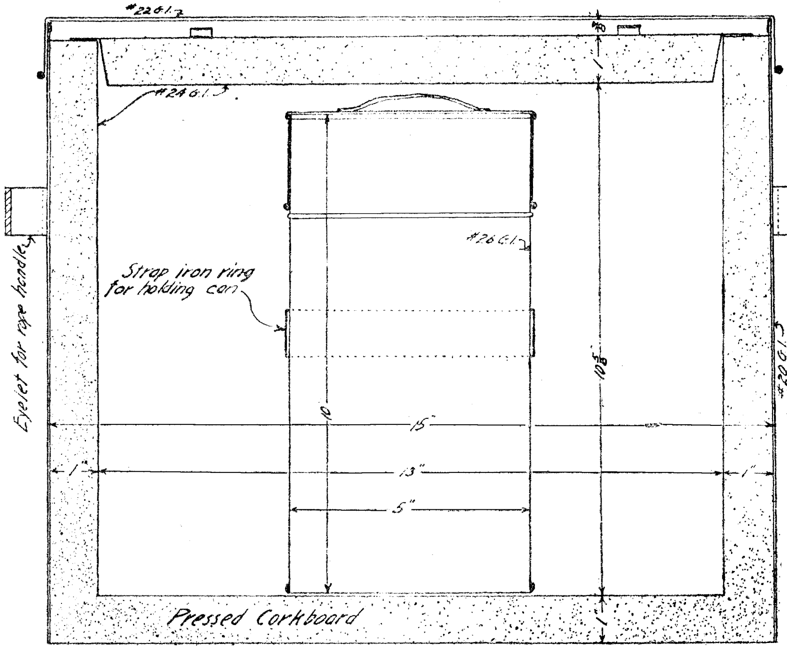

FIGURE 1.

case convenient to carry (see fig. 3). The inside can is just large enough to hold eight 4-ounce ground-glass stoppered sample bottles in two layers of four each.

As ordinarily shipped out, in the inner can are placed the eight bottles, protected from breakage by paper or cotton. Outside of the bottle can are placed eight descriptive blanks and eight manila envelopes, together with a shipping tag for the return of the case to the laboratory (see fig. 4).

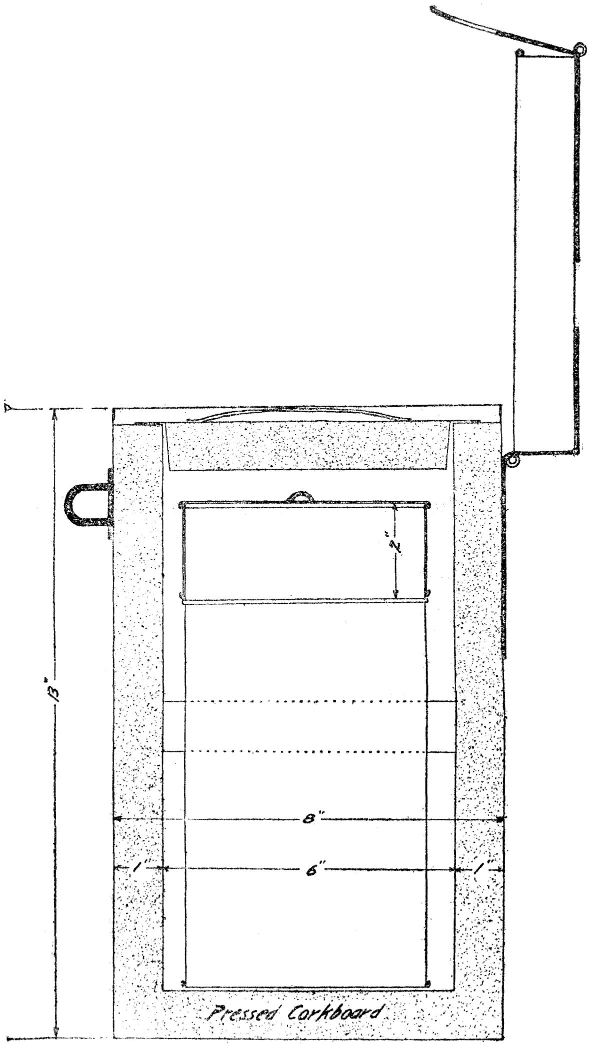

FIGURE 2.

When the sample is collected the descriptive blank is filled out and placed with the bottle in one of the envelopes, which is then placed

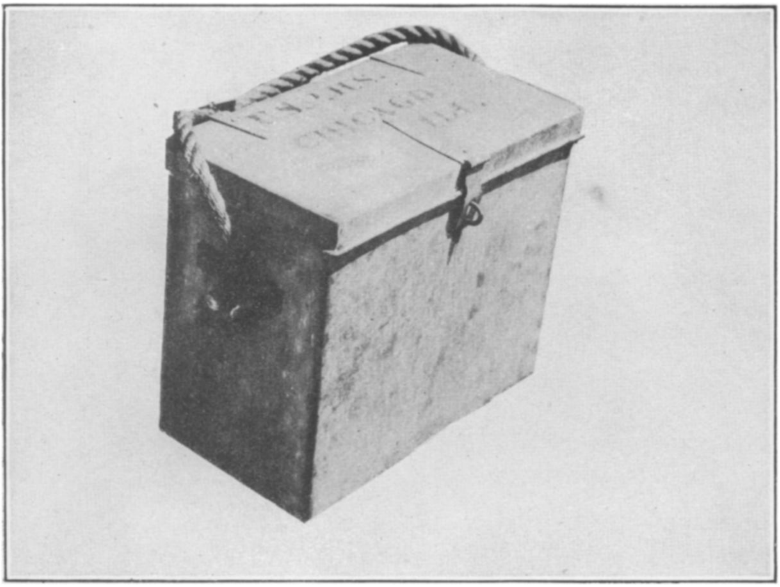

FIG. 3.—PHOTOGRAPH OF CASE.

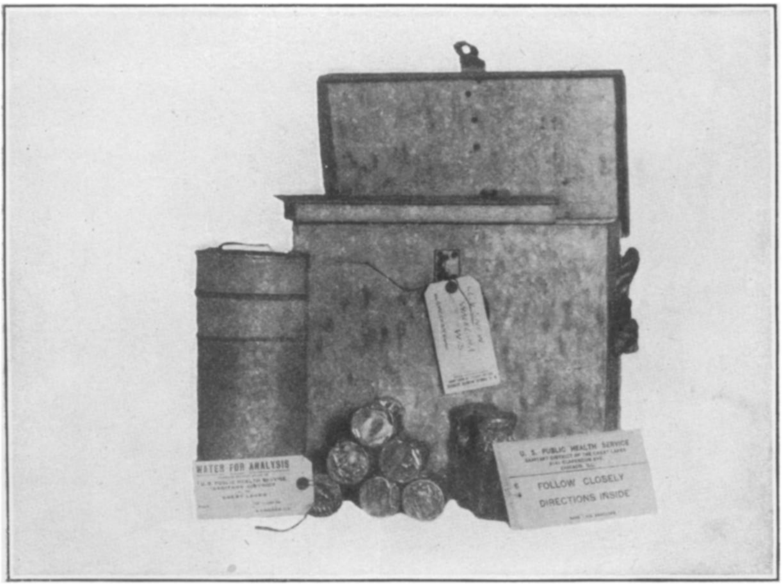

FIG. 4.—PHOTOGRAPH OF CASE AND CONTENTS AS SHIPPED OUT FROM LABORATORY.

within the inner can. The space around this can is filled with broken ice and requires about 13 pounds to completely fill the case.

Numerous tests have shown that the case will maintain eight 4-ounce samples at a temperature below 10° C. for a period of 48 hours.

The shape of the inner can is such that instead of the eight 4-ounce bottles, a liter bottle can be easily packed within it.

The first 25 cases purchased in 1915 cost complete $6.50 each. A similar lot purchased in 1917 cost $8 each, the additional cost being due to increases in the cost of galvanized iron.

In designing a shipping case, the importance of accomplishing the bacteriological examination of drinking water at the time of collection was fully appreciated. Immediate analysis, however, is not always practicable. In fact, at the present time a large proportion of all samples are not analyzed at once but are shipped to a central laboratory, either city or State. In order to minimize the bacterial change, all such samples should, of course, be well packed in ice. There is no doubt that this chilling process greatly lessens the probability of an erroneous interpretation, but, on the other hand, it is a well-known fact that the results are not entirely dependable, especially when the analysis is to be delayed a period of 24 hours or longer.

In the above connection, the 1917 issue of the "Standard Methods of Water Analysis" of the American Public Health Association gives the following directions: "The time allowed for storage or transportation of a bacterial sample between the filling of a sample bottle and the beginning of the analysis should be not more than six hours for impure waters and not more than 12 hours for relatively pure waters. During the period of storage the temperature shall be kept as near 10° C. as possible. Any deviation from the above limits shall be so stated in making reports."

A knowledge of the changes that take place both in the total count and the *B. coli* content of waters when kept for various lengths of time under different conditions is certainly a matter of great importance.

The results of Whipple's experiments to determine the changes in total count that take place in stored waters caused him to draw the following conclusions: "After the collection of a sample in either a large or a small bottle, there is a slight reduction in the number of colonies that can be obtained upon the gelatin plate, due apparently to the effect of changed environment upon the bacteria present. This reduction is perhaps somewhat greater when a small volume of water is collected, as its temperature change is naturally more rapid. The subsequent growth takes place more rapidly when the bottle is but partially filled than when it is completely full. With bottles of the same size the growth is more rapid in small volumes of water than in large volumes. The explanation of this is not wholly clear, but undoubtedly the supply of oxygen is an important factor, and it is probably the controlling factor." In his experiments the changes in coli content were not considered.

Jordan and Irons have shown that considerable changes take place in the total count of ice-packed samples, even within a few hours, and state that "The initial temperature determines to a large extent the course of events. * * * The influence of ice packing upon such colon bacilli as may be present in polluted water does not appear to be injurious. We have not been able to observe any marked disappearance of *B. coli*, either in ice-packed waters or in those maintained at a higher temperature during a period such as may ordinarily lapse during transportation." In a later publication Jordan states that "Our own experiments are too few in number to warrant generalization, but so far as they go, they indicate that no material change occurs in ice-packed samples within 48 hours, a period longer than that usually consumed in transportation."

Since the work of this district required long periods of storage, investigations were carried on to determine just what changes actually occurred in the bacterial content of the water. Observations were tabulated on some 30 series of water samples which contained or were inoculated artificially with strains of *B. coli*. The organisms selected for inoculation were obtained by fishing characteristic colonies from Endo's medium.

The three tables given below are in some degree typical of the results in all cases. However, the conclusions are based on the entire series.

Table No. 1.

A portion of a colony of *B. coli* was emulsified in 10 cubic centimeters of normal salt solution, and 1 liter of sterile distilled water was inoculated with 2 drops of this emulsion. The water was then thoroughly shaken to give an even distribution of the organisms and apportioned into nine sterile 4-ounce ground-glass-stoppered bottles. The water from one of these was planted immediately as control (sample C–5). Four others (C–6, C–7, C–8, and C–9) were placed in the 37° C. incubator and the remaining four (C–10, C–11, C–12, and C–13) kept on ice at a temperature below 6° C.

Sample No.	Time of analysis.	Total count on agar at 37° C.	Fermentation of lactose broth and confirmation on endo.						
			0.00001 c. c.	0.0001 c. c.	0.001 c. c.	0.01 c. c.	0.1 c. c.	1 c. c.	10 c. c.
C-5	Immediately	64,000	−	+	+	+	+	+	+
Kept at 37° C.:									
C-6	24 hours later	44,500	−	−	−	−	−	+	+
C-7	48 hours later	53,700	−	−	−	−	−	−	−
C-8	72 hours later	13,300	−	−	−	−	−	+	+
C-9	96 hours later	6,000	−	−	−	−	−	−	−
Kept below 6° C.:									
C-10	24 hours later	49,000	−	−	+	+	+	+	+
C-11	48 hours later	30,000	+	+	+	+	+	+	+
C-12	72 hours later	33,500	−	−	−	+	+	+	+
C-13	96 hours later	1,250	−	−	−	+	+	+	+

The colonies developing on agar plates from the incubated samples were quite small, even after several days' incubation. The colonies from the corresponding iced samples were large and healthy in appearance, resembling the colonies from the control sample (C-5).

The loss of fermenting power in dilutions of 0.1 c. c. and lower is noted, although the total number of viable organisms remains high. Such a result seems to demonstrate the sensitivity of the *B. coli* group to a 37° C. temperature in the absence of food. Likewise, under the same food conditions, the preserving power of ice, as far as fermenting quality is concerned, is shown in samples C-10 to C-13.

Table No. 2.

About two liters of Chicago tap water, known to contain small amounts of *B. coli*, were thoroughly shaken and distributed into eleven sterile four-ounce bottles. One sample was planted immediately, five samples were kept at room temperature (20° C.), and five below 6° C.

Sample No.	Time of analysis.	Total count on agar at 37° C.	Fermentation of lactose broth and confirmation on endo.		
			0.1 c. c.	1 c. c.	10 c. c.
C-100	Immediately	13	−	−	5+
Kept at room temperature:					
C-101	24 hours later	60	−	+	5+
C-102	48 hours later	65	−	−	4− 1+
C-103	72 hours later	34	−	−	4− 1+
C-104	96 hours later	130	−	−	5−
C-105	120 hours later	15	−	−	5−
Kept below 6° C.:					
C-106	24 hours later	14	−	+	5+
C-107	48 hours later	18	−	−	5+
C-108	72 hours later	26	−	−	4+ 1−
C-109	96 hours later	39	−	−	2+ 3−
C-110	120 hours later	34	−	−	5−

The samples kept at room temperature showed a slight increase in total count up to the fifth day, while in the ice-kept samples there was practically no change.

B. coli was demonstrated in the samples kept at room temperature in only one of five 10 c. c. portions of water on the third day and was entirely absent on the fourth and fifth days.

In the ice-kept samples *B. coli* was present in two 10 c. c. portions until the fourth day and absent on the fifth day.

Table No. 3.

About one liter of lake water, after being thoroughly shaken, was distributed into seven sterile four-ounce bottles. One was planted as control, two were kept below 6° C., two at room temperature, and two at 37° C.

Sample No.	Time of analysis.	Total count on agar at 37° C.	Fermentation of lactose broth and confirmation on endo.			
			0.01 c. c.	0.1 c. c.	1 c. c.	10 c. c.
C-252	Immediately	60	−	+	+	+
Kept below 6° C.:						
C-253	24 hours later	170	−	−	+	+
C-254	48 hours later	140	−	+	+	+
Kept at room temperature:						
C-255	24 hours later	340	−	−	+	+
C-256	48 hours later	5,000+	−	−	+	+
Kept at 37° C.:						
C-257	24 hours later	5,000+	−	−	−	+
C-258	48 hours later	10,000+	−	−	−	+

The above table shows a marked increase in total count that usually takes place in stored waters that are not chilled. A decrease in *B. coli* content somewhat indirectly proportionate to the temperature at which the samples were kept is noted.

No consistent increase of the *B. coli* content was noted in the entire series of 30 tests, regardless of the conditions under which the water was kept.

On four occasions a slight increase was indicated by the fermentation of lactose broth, but it is to be recalled that this is not conclusive, since the methods of dilution which are necessarily employed to carry out the fermentation tests are only roughly accurate.

Usually there was a marked decrease of *B. coli* in natural samples of water kept at 37° C. and room temperature. Ice-kept samples also showed a slight decrease in *B. coli*. It is a recognized fact that *B. coli* out of its natural environment will die out. This is more marked when the temperature is above 20° C.

The changes that took place in the total count were very irregular, and the indications are that neither an increase nor a decrease can be predicted. Low temperatures exercised an inhibiting effect, and usually enormous increases occurred in all samples not packed in ice. Erratic and unreliable results are to be expected since there are so many varieties of organisms that might be present in any given sample. On the other hand, one family or group such as the *B. coli.* might be reasonably expected to follow very nearly the same biological changes when the samples are subjected to the same conditions during the period between collection and analysis. The above tests indicate that this is the case in delayed samples that have been kept below 10° C. Such a sample could reasonably be stated to have had *B. coli* present at the time of collection in at least the amounts present at the time of analysis, and very likely in greater amounts. If *B. coli* were absent on analysis, it was probably absent when the sample was collected. However, it may have been present in very small amounts and have died out during the period of storage.

The results obtained in these experiments are in conformity with what little has been published by other workers along the same line. In view of the enormous number of analyses made on shipped samples, the necessity of knowing what changes take place during storage is of great importance, and further study along these lines is suggested as a profitable line of investigation.